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ORIGINAL ARTICLE

Epidemiologic Study of Diarrhea Pathogens Detected by Multiplex Real-Time PCR: a Single Center Study During 1 Year

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SUMMARY

Background: **Acute gastroenteritis is one of the major causes of morbidity and mortality worldwide, especially in children and the elderly. The identification of various diarrhea-causing bacteria using multiplex polymerase chain reaction (PCR) and rapid antigen testing has enabled a more detailed analysis of diarrhea-causing pathogens. Previous reports have a limitation in that they do not include data on multiple infections in which two or more infectious agents are simultaneously detected, and there are no data on clinical information. We investigated various diarrhea-causing bacteria and viruses detected by multiplex real-time PCR for one year at a single institution.**
Methods: **This study included 766 subjects who underwent multiplex real-time PCR testing of direct stool specimens for the purpose of diagnosis from April 2019 to February 2020. The multiplex PCR test used in our study can simultaneously detect 16 types of bacteria and five types of viruses. When two or more pathogens were detected by multiplex real-time PCR, they were confirmed using single conventional PCR or real-time PCR. Demographic, clinical, and laboratory data were collected from electronic medical records (EMR). The detected bacteria and viruses were analyzed according to age and season.**
Results: **Out of a total of 352 stool samples with pathogen detection, 265 (75.3%) were detected as single and 87 (24.7%) showed co-detection. The highest rates of single and co-detection were for *Clostridium perfringens,* and the highest combination of co-infections was for *C. perfringens* and *Staphylococcus aureus*.**
Conclusions: **We demonstrated that different age groups showed varying pathogen distributions. While no special seasonality was found in the monthly distribution, it should be noted that the total number of cases peaked in September. The data presented in our study serves as epidemiologically important basic data.**
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Supplementary Data

Table S1.1. Conventional PCR for the detection of EPEC, EIEC, EAEC, and ETEC.
*** Primer information (Final concentration 500 nM, each).**

Target			Sequence	Size (bp)
EPEC	eaeA	F	CCC GGA TTC GGC ACA AGC ATA AGC	881
		R	CCC GGA TCC GTC TCG CCA GTA TTC G	
EIEC	inV	F	TTT CCC TCT TGC CTG CAT ATG CGC	465
		R	CTC ACC ATA CCA TCC AGA AAG AAG	
EAEC	aggR	F	CTA ATT GTA CAA TCG ATG TA	457
		R	AGA GTC CAT CTC TTT GAT AAG	
ETEC	LT	F	GCA CAC GGA GCT CCT CAG TC	218
		R	TCC TTC ATC CTT TCA ATG GCT TT	
	ST	F	TCA CCT TTC CCT CAG GAT GC	179
		R	ATA TTA TTA ATA GCA CCC GG	
	STp	F	TCT TTC CCC TCT TTT AGT CAG	165
		R	ACA GGC AGG ATT ACA ACA AAG	

*** PCR condition:**
1) EAEC.

Step	Temperarture (℃)	Time	Cycle number
Initial denaturation	95	15 minutes	1
Cycling stage	94	1 minute	30
	55	1 minute	
	72	1 minute	
Elongation	72	10 minutes	1
Storage	4	-	-

2) ETEC (*STp*).

Step	Temperarture (℃)	Time	Cycle number
Initial denaturation	94	5 minutes	1
Cycling stage	94	30 seconds	30
	60	30 seconds	
	72	1 minute	
Elongation	72	10 minutes	1
Storage	4	-	-

3) EPEC, EIEC, ETEC (*LT*, *ST*).

Step	Temperarture (℃)	Time	Cycle number
Initial denaturation	95	5 minutes	1
Cycling stage	95	30 seconds	35
	50	40 seconds	
	72	1 minute	
Elongation	72	10 minutes	1
Storage	4	-	-

Table S1.2. Real-time PCR for the detection of *B. cereus, Salmonella spp., Y. enterocolitica, C. perfringens, S. aureus*, Norovirus GI, GII, astrovirus, and adenovirus.
*** Primer, probe information.**

Target			Sequence	Final Conc. (nM)
Bacillus cereus	16S rRNA	F	GCG GCG TGC CTA ATA CAT GC	500
		R	CTC AGG TCG GCT ACG CAT CG	500
		P	FAM-TCG AGC GAA TGG ATT AAG AGC TTG C - [illegible]	100
Salmonella spp.	*invA*	F	GAA TCC TCA GTT TTT CAA CGT TTC	500
		R	CGA ATT GCC CGA ACG TGG CGA	500
		P	FAM - CTC TTT CGT CTG GCA TTA TCG ATC AGT ACC AG - BHQ1	100
Yersinia enterocolitica	*ail*	F	ATG ATA ACT GGG GAG TAA TAG GTT CG	250
		R	CCC AGT AAT CCA TAA AGG CTA ACA TAT	250
		P	FAM-TCT ATG GCA GTA ATA AGT TTG GTC ACG GTG ATC T - BHQ1	100
Clostridium perfringens	*cpa*	F	AAA AGA AAG ATT TGT AAG GCG CTT AT	500
		R	CCC AAG CGT AGA CTT TAG TTG ATG	500
		P	FAM-TGC CGC GCT AGC AAC TAG CCT ATG G - [illegible]	250
Staphylococcus aureus	*femA*	F	AAT AAT AAC GAG GTC ATT GCA GCT T	250
		R	TGG ACC GCG ATT TGA ATA AAA	250
		P	FAM-CTT ACT TAC TGC TGT ACC TGT T - MGB	100
Norovirus GI, GII	COG1F	F	CGY TGG ATG CGN TTY CAT GA	250
	COG1R	R	CTT AGA CGC CAT CAT CAT TYA C	250
	RING1(a)-TP	P	FAM-AGA TYG CGA TCY CCT GTC CA - TAMRA	100
	BPO-13	F	AIC CIA TGT TYA GIT GGA TGA G	250
	BPO-13N	F	AGT CAA TGT TTA GGT GGA TGA G	250
	BPO14	R	TCG ACG CCA TCT TCA TTC ACA	250
	BPO18	P	VIC-CAC RTG GGA GGG CGA TCG CAA TC - TAMRA	100
Astrovirus	AstV	F	CCD GCC AGR CTC ACA GAA GAG	250
		R	GAC TTG CTA GCC ATC ACA CTY C	250
		P	FAM-ACT CCA TCG CAT TTG GAG GGG AGG ACC - TAMRA	100
Adenovirus	ADV	F	TCG ATG ATG CCG CAR TG	250
		R	AGG CCC GGG CTC AGR TAC T	250
		P	FAM-ATG CAC ATC GCC GGG CAG GAC G - TAMRA	100
Rotavirus	NVP3	F	ACC ATC TWC ACR TRA CCC TC	250
		R	GGT CAC ATA ACG CCC CTA TA	250
		P	FAM-ATG AGC ACA ATA GTT AAA AGC TAA CAC TGT CAA - TAMRA	100

*** PCR condition:**
1) *B. cereus, Salmonella spp., Y. enterocolitica, C. perfringens, S. aureus.*

Step	Temperarture (°C)	Time	Cycle number
Polymerase activation	50	2 minutes	1
Denaturation	95	10 minutes	1
Cycling stage	95	15 seconds	40
	60 *	1 minute	

2) Norovirus, Astrovirus, Adenovirus, Rotavirus.

Step	Temperarture (℃)	Time	Cycle number
Polymerase activation	**45**	**30 minutes**	**1**
Denaturation	**95**	**10 minutes**	**1**
Cycling stage	**95**	**15 seconds**	**45**
	56 *	**1 minute**	

Table S1.3. Real-time PCR for the detection of *V. parahaemolyticus, C. jejuni, and C. coli.*
* **Primer, probe information.**

Target	Sequence			Final conc.
Vibrio parahaemolyticus	***toxR***	**F**	**GAA CCA GAA GCG CCA GTA GT**	**500**
		R	**AAA CAG CAG TAC GCA AAT CG**	**500**
		P	**FAM-TCA CAG CAG AAG CCA CAG GTG C - TAMRA**	**100**
Campylobacter jejuni	***mapA***	**F**	**CTG GTG GTT TTG AAG CAA AGA TT**	**300**
		R	**CAA TAC CAG TGT CTA AAG TGC GTT TAT**	**300**
		P	**FAM-TTG AAT TCC AAC ATC GCT AAT GTA TAA AAG CCC TTT - TAMRA**	**250**
Campylobacter coli	***ceuE***	**F**	**AAG CTC TTA TTG TTC TAA CCA ATT CTA ACA**	**300**
		R	**TCA TCC ACA GCA TTG ATT CCT AA**	**300**
		P	**VIC-TTG GAC CTC AAT CTC GCT TTG GAA TCA TT - TAMRA**	**250**

* **PCR condition:**

Step	Temperarture (℃)	Time	Cycle number
Polymerase activation	**50**	**2 minutes**	**1**
Denaturation	**95**	**10 minutes**	**1**
Cycling stage	**95**	**15 seconds**	**40**
	60 *	**1 minute**	